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# Potential Tetany Hazard of N-Fertilized Bromegrass as Indicated by Chemical Composition

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## ABSTRACT

The objective of this field experiment was to determine the effect of N fertilization on yield and chemical composition of smooth bromegrass (*Bromus inermis* L.) and the potential for grass tetany hazard in the northern Great Plains as indicated by chemical composition of bromegrass forage. Chemical components of forage considered in relation to the hazard of tetany (a metabolic disorder of ruminants resulting from forage with low Mg availability) were inorganic cations, organic anions, aconitate, and % total N/% total water-soluble carbohydrate ratio (N/TWSC). Soil was Parshall fine sandy loam, a pachic haploborall. Yields and chemical composition of oven dried forage from plots not previously harvested were determined at approximately 3-week intervals beginning May 9. Differences between the sum (in meq/kg) of inorganic cations ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$) and inorganic anions ($Cl^-$, $No_3^-$, $H_2PO_4^-$, $SO_4^{2-}$) in forage was defined as the concentration of organic anions (C-A).

Mature forage yield obtained from the unfertilized check plot treatment on July 29 was only 29 and 22% of yields obtained from plot treatments fertilized with 90 and 270 kg N/ha, respectively. The K/(Ca+Mg) ratios and K concentrations increased during May and early June, resulting in a K/(Ca+Mg) ratio near or above 2.2 during June and early June in oven dried forage from fertilized treatments. Potassium, expressed as a fraction K/C of the total cations (C), accounted for 35 to 74% of the cationic charge. Fertilization with N increased total N and K concentration and K/C in the forage. As K/C increased, Mg/C and Ca/C decreased and K/(Ca+Mg) increased. Aconitate and C-A concentration correlated highly with K concentration and were increased by N fertilization. Aconitate levels exceeded 1% on May 28; the 270 kg N-treatment remained above 1% through July. Nitrogen fertilizer increased N/TWSC in spring-harvested forage, compared to unfertilized forage, and greatly accentuated the peak N/TWSC values occurring in late spring samples.

This study indicated that although potential for increased forage and livestock-carrying capacity with N fertilization is tremendous, N-fertilization may result in a potential tetany hazard to ruminants. Therefore, management practices are needed which minimize tetany hazard while bromegrass yields are increased by N fertilization.


*Additional index words:* Hypomagnesemia, Grass tetany, Organic acids, Range, Forage, Organic Anions, Ionic balance, Carbohydrates.

ONLY recently have we recognized fertilization's tremendous potential for increasing productivity of both native and introduced species on western grasslands. Rogler and Lorenz (20) estimated that nitrogen fertilizer can be used successfully on approximately 24 million ha of grassland in the eastern part of the Great Plains of the United States. Although carrying capacity and total digestible nutrient (TDN) production are known to be increased by N fertilization, there is a general lack of information about changes in the chemical composition of forage species.

The mineral and organic composition of forage is important because of its relation to the occurrence of grass tetany, a metabolic disorder of ruminants resulting from forage with low availability of magnesium. Under field conditions, chemical composition of forage is affected by such weather conditions as temperature and precipitation which, in turn, influence the occurrence of grass tetany (10, 15). However, N fertilization can also have a large effect on the chemical composition of forage. Research in Nevada (15) and Ohio (2) has indicated that N fertilization increased Mg concentration in forage. Other reports (11, 13) from the Netherlands indicated that high concentrations of crude protein in forage increased the likelihood of tetany and decreased Mg in the blood serum of livestock.

Other chemical characteristics of forage associated with tetany are high levels of K (2.0 to 4.0%) (16), a ratio of K/(Ca + Mg) greater than 2.2 (14), *trans*-aconitic acid content greater than 1% (22), and a large and rapid increase in the % total N/% total water soluble carbohydrate ratio (N/TWSC) (15). Tetany may also be related to total organic acids (9, 10). For a detailed review of the tetany problem, the reader is referred to Grunes (9), and to Grunes et al. (10).

The objective of this study was to determine the effect of N-fertilization on chemical composition and yield of bromegrass (*Bromus inermis* L. cv. 'Lincoln') as the growing season progressed.

## METHODS AND MATERIALS

A uniform stand of established smooth bromegrass was used. Ammonium nitrate (33-0-0) was broadcast at three rates (0, 90, and 270 kg N/ha) on May 2, 1968. Concentrated superphosphate (0-46-0) was bloadcast uniformly with the N at the rate of 28 kg P/ha. Each treatment was replicated four times. Soil was Parshall fine sandy loam, a pachic haploborall, with a pH of about 6.5, no free $CaCO_3$ in the surface 30 cm, and a cation exchange capacity (CEC) of approximately 15 meq/100 g; the saturation extract had 0.3, 0.2, 2.0, and 1.1 meq/liter of Na, K, Ca, and Mg, respectively.

At each sampling date, a previously unharvested area 0.91 by 2.44 m was clipped at a 2.5-cm height. Harvested plant material was oven dried at 70 C, weighed, subsampled, and ground to pass a 20-mesh sieve for later chemical analysis. Concentrations of $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ were determined by atomic absorption spectrophotometry on plant material digested with $HNO_3$ and $HClO_4$. Phosphorus was determined colorimetrically using the vanadomolybdophosphoric acid method (12). Nitrates were determined by the phenoldisulfonic acid method (12). Total N, including $NO_3$-N, was determined by Kjedahl procedure (4). Chloride was determined in an extract by shaking 1 g of plant material for 30 min with 50 ml of 0.1 *N* formic acid, filtering, and potentiometrically titrating 25 ml of the solution with 0.01

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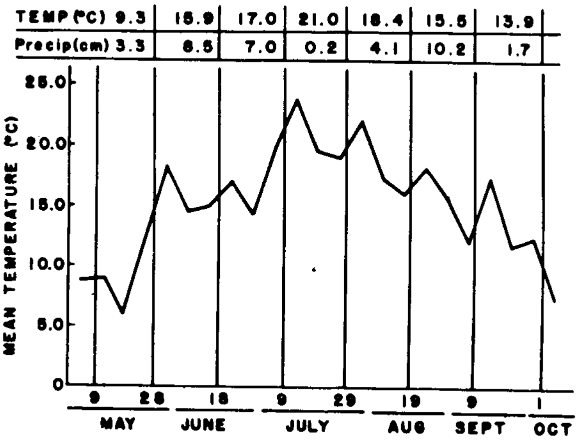


Fig. 1. Mean temperatures, harvest dates (vertical lines), and total precipitation between harvests.

N $AgNO_3$. Total S in the plant material was determined from a $HNO_3$ and $HClO_4$ digestion (3).

Organic acid concentration (C-A) was calculated as the difference between inorganic cation concentration ($C = Na^+ + K^+ + Ca^{2+} + Mg^{2+}$) and inorganic anion concentration [$A = NO^-_3 + Cl^- + SO^{2-}_4 + P$ as $H_2PO_4^-$ (23)]; all data were expressed as meq/kg dry matter. The $SO^{2-}_4$-sulfur in meq/kg present was estimated using analytical data for total N, $NO^-_3$-N, and total S as described by van Tuil (23). Total aconitate was determined by modifying a polarographic procedure (17). Percent total water-soluble carbohydrates (TWSC) were determined as reducing sugars (16).

## RESULTS AND DISCUSSION

*Temperature and Precipitation.* Mean weekly temperatures and harvest dates for the season and mean temperature and total precipitation between harvest dates are shown in Fig.1. A total of 35 cm of precipitation was recorded between the first and final harvests. Previous research (14) indicated increased incidence of grass tetany 5 days after mean daily temperatures rose above 14 C. In this study, mean temperatures above 14 C were observed from late May until early September.

*Yields.* A significant response in forage yield (oven-dried basis) as compared with check treatment was obtained from the high N treatment by May 28 (Table 1, Section A). On June 18 and thereafter both the 90 and 270-kg N treatment significantly increased yield over the check treatment. The 270 kg N treatment significantly increased yield over the 90 kg N treatment on July 29 and thereafter.

Dry matter accumulation rates were highest for the three growth periods between May 28 and July 29. Temperatures averaged 15.9, 17.0, and 21.0 C, respectively, during these three periods. Growth rate declined after July 9 as the plants approached maturity. A significant decrease in forage yields was observed between the July 29 and August 19 harvests with very little change in total dry matter thereafter.

*Total N.* Total-N concentration (Table 1, Section B) in forage (oven-dried basis) increased significantly above that of the check treatment by N fertilization at all harvest dates between May 9 and July 29. However, after July 29 only forage from the 270 kg N treatment plots had a significantly higher total N concentration than the check treatment. Forage from the 270 kg N treatment was higher in total N than that from the 90 kg N treatment at all harvests except the first. Total N in forage from fertilized treatments decreased after the May 28 harvest and tended to level out after about the July 29 harvest. Decreases in total N concentration in bromegrass forage during the late summer have been reported previously by Power (18) who related the degree of decline to the decrease in water availability.

*Inorganic Cations.* The K concentration in the forage increased most rapidly between May 9 and 28 (Table 1, Section C). During this period temperature also increased markedly. Concentration of K continued to increase between the May 28 and June 18 harvests. Thereafter, K concentration tended to decrease. Fertilization with N increased K concentration over the control treatment on all harvest dates after May 9. The 270 kg N treatment significantly increased K concentration of the forage over that of the 90 kg N treatment on all harvest dates after June 18.

Fertilization with N had no significant effect on Ca concentration in the forage until the September 9 and Ootcber 1 harvests, when forage from the check treatment had a significantly higher Ca concentration than forage from fertilized treatments (Table 1, Section D). Forage from the two fertilized treatments never differed significantly in Ca concentration throughout the season. Perhaps the most important aspect of Ca concentration was its rapid decrease at the June 18 harvest, when Ca concentration averaged only 54 and 62% and were significantly lower (99% confidence level) than those measured on May 9 and 28, respectively. The lowest Ca concentration measured was 142 and 141 meq Ca (0.28%), on July 9 and 29, respectively, for the 90 kg N/ha rate. This is within the 0.27 to 0.29% Ca range recommended for beef cows nursing calves, first 3 to 4 months postpartum (6). Bromegrass pastures in the Northern Great Plains are expected to be used primarily for beef cattle. However, the Ca is well below the 0.40 to 0.45% Ca recommended in the Netherlands (11) for moderately productive dairy cows with annual milk productions of 4,000 to 4,500 kg. For highly productive dairy cows producing 6,000 kg milk annually, the recommended Ca concentration in the feed should be 0.60% (11).

Magnesium concentrations in the forage (Table 1, Section E) were highest at the first harvest date averaging 146 meq/kg (0.18%). A Mg level of 0.20% (164 meq/kg) has been suggested as the concentration above which tetany seldom occurs unless percent N or K are high (9, 10, 11, 13, 16). Therefore, in this study the Mg concentrations in forage approach those considered as tetany prone, especially when the high N and K levels in the forage are also considered. Average Mg level in the forage at the June 18 harvest was 76 and 90% of that measured on May 9 and 28, respectively. The check and 270 kg N treatment did not have significantly different levels of Mg in the forage at any date, but both were significantly higher than the 90 kg N treatment beginning on July 9 and tended to remain higher thereafter. As noted by the increases in forage production (Table 1, Section A) Mg was apparently diluted more by the 90 kg N treatment than for the 270 kg N treatment where Mg uptake was relatively greater.

*Cation Ratios.* Tetany cases are reportedly more frequent when the ratio of K/(Ca + Mg) on a meq basis exceeds 2.2 (9, 10). The K/(Ca + Mg) ratio in this study (Fig. 2A) increased rapidly between the May 9 and June 18 harvests, at which time ratios for all treatments exceeded 2.2. Air temperature (Fig. 1) and forage yield (Table 1, Section A) also increased during this time. Forage from both fertilized treatments had significantly higher K/(Ca + Mg) ratios compared with the check at all harvest dates except the first, but between N levels the ratio was not significantly different until the September 9 and October 1 harvests. Forage from fertilized treatments had a K/(Ca + Mg) ratio near or above 2.2 for at least 6 weeks during June and early July, a period during which bromegrass would normally be grazed by livestock.

Comparison of K data (Table 1, Section C) with the K/(Ca + Mg) ratio (Fig. 2A) indicates that a measurement of the K concentration alone would not adequately describe the K/(Ca + Mg) relationship. For example, K concentrations increased at a relatively slower rate between the May 28 and June 18 harvests than did K/(Ca + Mg) because Ca concentrations, and to a lesser extent Mg concentrations, decreased as dry matter production rapidly increased between May 28 and June 18. Also K concentration of fertilized treatments decreased at a relatively faster rate between the June 18 and July 29 harvests than did K/(Ca + Mg) ratios.

Follett and Reichman (8), in a growth room experiment, suggested that accumulated organic anions in plant material may largely be present as K salts. Of the cations measured in the present study, K accounted for a large percentage of the cationic charge available to satisfy charge associated with inorganic and organic anions. Fig. 2B shows the ratio of K to organic plus inorganic anions [K/(C—A) + A = K/C] as expressed as a percentage.

Nitrogen fertilization significantly increased K/C at all harvests except the first. The 270 kg N treatment did not differ significantly in K/C from the 90 kg N treatment until the October 1 harvest, and did not differ significantly from the check treatment at the third harvest.

For plants grown in soil, cations such as Fe, Mn, Zn, and Cu are absorbed in very small quantities as compared with total uptake of K, Na, Ca, and Mg and in most plants absorbed $NH_4$ is completely metabolized (23). Thus, with low Na concentration, K + Ca + Mg account for nearly all of the inorganic cation concentration (C). Consequently, the relationship of K/C to K/(Ca + Mg) is $\frac{1}{K/C} - 1 = \frac{1}{K/(Ca + Mg)}$. When K/(Ca + Mg) is 2.2, K accounts for 69% of the cationic charge (C). In forage containing 1,000 meq of cations/kg, K content will therefore be 2.7% when K/(Ca + Mg) = 2.2. If K/C increases above 0.69 (69%), the K/(Ca + Mg) ratio increases very rapidly, reaching 4 when K/C = 0.80 (80%).

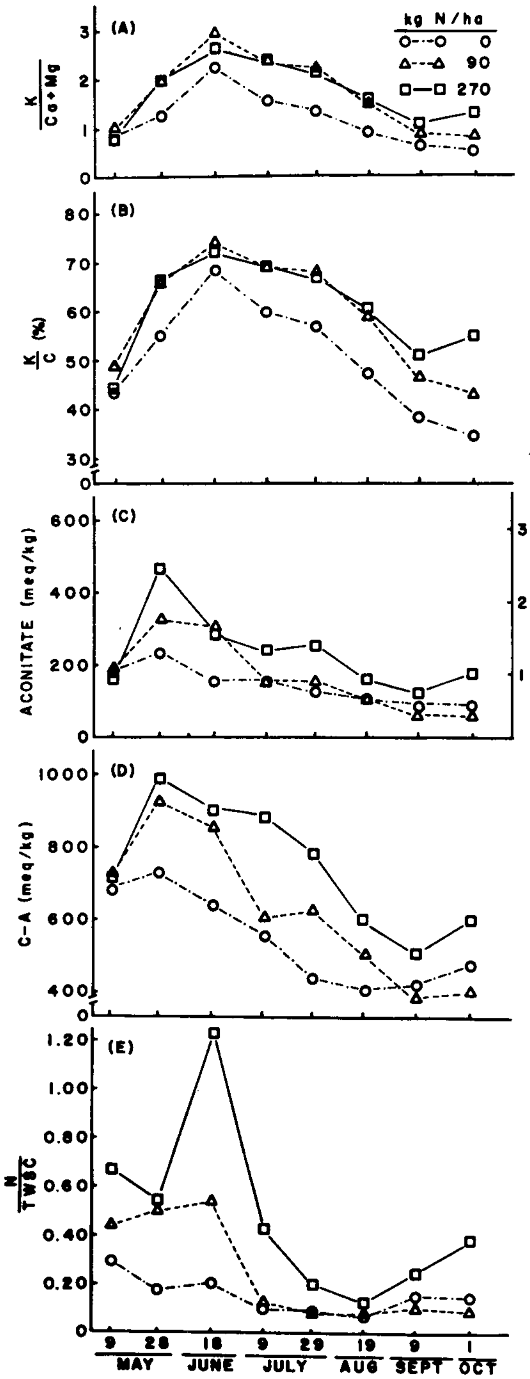


Fig. 2. The effect of N fertilization of bromegrass as a function of date of harvest on: (A) K/(Ca+Mg) ratio, (B) meq/kg of K as a % of meq/kg of cations (C = $K^+$ + $Na^+$ + $Ca^{2+}$ + $Mg^{2+}$), (C) aconitate concentration, (D) organic anion (C-A) concentration, and (E) % total N/% total water-soluble carbohydrate ratio in oven-dried forage.

*Estimates of Blood Mg.* Another method of assessing tetany hazard of forage is described by Henkens (11), who used the Mg, N, and K content in forage to graphically estimate Mg concentration in cattle's blood serum. According to Henkens (11), estimated blood serum Mg decreases as Mg content of grass decreases and as the product of %K and crude protein

Table 1. Bromegrass yield and chemical composition by harvest date and N rate.

| | May | | June | July | | Aug. | Sept. | Oct. |
|---|---|---|---|---|---|---|---|---|
| N Rate | 9 | 28 | 18 | 9 | 29 | 19 | 9 | 1 |
| kg/ha | | | | | | | | |
| | | | | Section A. Oven dry forage Yield, kg/ha | | | | |
| 0 | 144 | 376 | 692 | 1,292 | 1,658 | 1,231 | 1,200 | 1,088 |
| 90 | 212 | 885 | 3,011 | 5,259 | 5,656 | 4,852 | 4,730 | 4,933 |
| 270 | 291 | 1,052 | 3,382 | 6,652 | 7,385 | 5,706 | 6,021 | 6,144 |
| L. S. D. 0.05 | ns | 521 | 780 | 1,706 | 1,182 | 779 | 574 | 831 |
| L. S. D. 0.01 | ns | ns | 1,182 | 2,584 | 1.791 | 1,180 | 870 | 1,259 |
| | | | | Section B. Total N, % | | | | |
| 0 | 1.76 | 1.64 | 1.31 | 1.21 | 0.82 | 0.93 | 1.02 | 1.01 |
| 90 | 2.43 | 3.52 | 2.35 | 1.42 | 1.04 | 0.96 | 0.90 | 0.82 |
| 270 | 2.46 | 4.27 | 3.26 | 2.31 | 1.68 | 1.47 | 1.43 | 1.47 |
| L. S. D. 0.05 | 0.51 | 0.35 | 0.35 | 0.24 | 0.20 | 0.14 | 0.25 | 0.35 |
| L. S. D. 0.01 | ns | 0.53 | 0.52 | 0.37 | 0.30 | 0.22 | 0.38 | 0.52 |
| | | | | Section C. K, meq/kg* | | | | |
| 0 | 368 | 518 | 571 | 446 | 349 | 276 | 225 | 219 |
| 90 | 432 | 708 | 764 | 512 | 492 | 354 | 225 | 217 |
| 270 | 387 | 777 | 806 | 720 | 610 | 438 | 307 | 388 |
| L. S. D. 0.05 | ns | 82 | 44 | 68 | 82 | 96 | 58 | 78 |
| L. S. D. 0.01 | ns | 124 | 67 | 103 | 124 | ns | ns | 119 |
| | | | | Section D. Ca, meq/kg** | | | | |
| 0 | 316 | 289 | 153 | 187 | 161 | 187 | 233 | 294 |
| 90 | 296 | 247 | 156 | 142 | 141 | 158 | 167 | 187 |
| 270 | 314 | 268 | 187 | 201 | 178 | 176 | 182 | 208 |
| L. S. D. 0.05 | ns | ns | ns | ns | ns | ns | 29 | 30 |
| L. S. D. 0.01 | ns | ns | ns | ns | ns | ns | 44 | 45 |
| | | | | Section E. Mg, meq/kg† | | | | |
| 0 | 138 | 125 | 107 | 107 | 101 | 111 | 125 | 115 |
| 90 | 146 | 119 | 107 | 82 | 84 | 82 | 86 | 84 |
| 270 | 154 | 127 | 119 | 117 | 115 | 105 | 109 | 99 |
| L. S. D. 0.05 | ns | ns | ns | 17 | 20 | 18 | ns | 19 |
| L. S. D. 0.01 | ns | ns | ns | 26 | ns | ns | ns | ns |

* K (%) = K (meq/kg) × $3.91 \times 10^{-3}$)   ** Ca (%) = Ca (meq/kg) × $2.00 \times 10^{-3}$)   † Mg (%) = Mg (meq/kg) × $1.22 \times 10^{-3}$)

(6.25 × %N) content increase. Dietary supplimentation with Mg may be necessary when Mg content in blood serum approaches 20 ppm or less (11).

Estimates of Mg concentration of blood serum were made using chemical data from the present study (Table 1) and the graph shown on page 15 in Henkens (11). Estimates of concentration of blood serum Mg from the 90 kg N treatment were 13 and 18 ppm on the May 28 and June 18 harvests, respectively, whereas from the 270 kg N treatment they were 6 and 14 ppm for the two dates. For all other harvests of the 90 and 270 kg N treatment as well as all harvests from the check treatment, forage gave estimated values greater than 20 ppm in blood serum. Thus, N fertilization decreased estimated blood serum Mg, and the intensity of the tetany hazard was estimated as greater with application of 270 than with 90 kg N/ha. With Henkens's (11) method, May 28 and June 18 may have been the most hazardous period of time for tetany. May 28 was approximately the time when mean daily temperature rose above 14 C (Fig. 1), a temperature rise which previous research (14) has shown corresponds with increased incidence of tetany.

*Organic Acids.* Although total aconitate (*cis-* and *trans*-forms) were measured in this study, previous research by Burau (5), Prior et al. (19), and Stout et al. (22) indicated most aconitic acid in grasses is present in the *trans*-form. Highest concentrations of aconitate were obtained at the May 28 harvest for all three treatments, after which they generally declined (Fig. 2C). If the potential toxication level is 1.0% (22), then even the nonfertilized control treatment may pose some hazard to cattle during May or early June (1% aconitate equals 172 meq aconitate/kg plant material). The 90 kg N treatment increased aconitate only early in the season, while the 270 kg N treatment increased aconitate levels throughout the season as compared with the control treatment. The 270 kg N treatment produced forage with 2.7% aconitate on May 28 and identifies a potential tetany hazard from N-fertilization. Barta (1) had found that N fertilization increased concentrations of aconitic acid in bromegrass. He indicated that concentration of malic and citric acids increased as well.

The difference between the inorganic cation and inorganic anion (C—A) content, as described by DeWit, Dijkshoorn, and Noggle, (7) is generally accepted as a reliable estimate of the organic acid concentration. Livestock intake of forages high in organic acids may result in chelation of Ca and Mg, which affects normal transference pathways of Ca and Mg within ruminants (22). Concentrations of C—A were highest at the May 28 harvest (Fig. 2D) and generally decreased thereafter, except at the final harvest. Nitrogen fertilizer, particularly at 270 kg N/ha, increased C—A above the check treatment for all harvests except the last two. At the October 1 harvest, the 90 kg N-treatment decreased C—A significantly below that of the check.

Previous research (21) indicates a high correlation between oxalic acid content of three *Setaria sphacilata* cultivars and C—A, and that K and N fertilization increased oxalic acid content. Although oxalic acid content was not measured in the present study on bromegrass , meq/kg of aconitate correlated highly with C—A during the entire growing season for all N-treatments [aconitate = 0.49 (C—A — 131, r = 0.89**]. The slope of 0.49 indicates that as C—A increases, other organic acids besides aconitate simultaneously increase. The fraction of total organic acid (C—A) in the form of aconitate averaged 27% for all harvests and treatments. However, a maximum value of 47% aconitate resulted from the 270 kg N-treatment on May 28 which coincided with peak C—A and total N values, just before peak values for K and K/(Ca + Mg) occurred on June 18. May 28 was also the date when lowest estimated values of blood serum Mg occurred for the N-fertilized treatments.

As with cationic relationships, K also seems to be strongly involved with anionic relationships. In this study, for all treatments and harvests dates, meq/kg of C—A was highly correlated with meq/kg of K; [C—A = 0.90 K + 226, r = 0.92**]; constants in this equation are similar to those reported previously by Follett and Reichman (8) for barley [C—A = 1.05 K + 268, r = 0.97**], also grown on Parshall fine sandy loam, but in a growth room with P fertility, water, and soil temperature variables. Slopes of 0.90 and 1.05 in the above relationships of C—A to K indicate increases of organic acid concentration were largely in the form of K-salts. In the present study, the total season relationship of meq/kg of aconitate to meq/kg of K was [aconitate = 0.45 K − 25, r = 0.85**]. The slope of 0.45 indicates that organic acids simultaneously increased as K increased. A level of 1% (172 meq/kg) aconitate might be observed with a K concentration in the range of 440 meq/kg (1.72%) K; or K/C = 0.44 (44%) and K/(Ca + Mg) = 0.78, if C is 1000 meq/kg.

Forage N/TWSC imbalance occurs when environmental conditions produce rapid forage growth. Fertilizer N enhances this rapid growth rate and further depletes the supply of TWSC in the plant while at the same time providing luxuriant uptake of N. Responses of N/TWSC ratios to N fertilization like those shown in Fig. 2E have been reported (15) in crested wheatgrass (*Agropyron desertorum*) with N/TWSC ratio peaks coinciding with occurrence of grass tetany. Rapid increase in forage N/TWSC ratio is hypothesized to produce conditions in ruminants which reduce dietary Mg availability (15, 16).

The N/TWSC ratios measured in forage from the present study were highest for the 270 kg N treatment plots, intermediate for the 90 kg N treatment plots, and lowest for unfertilized treatment plots (Fig. 2E). The 90 kg N treatment plots were not significantly different in N/TWSC ratio from the check treatment plots after July 9. Peaks in the N/TWSC ratio of N fertilized forage occurred on June 18 and coincided with peak values for the K/Ca + Mg and K/C ratios but followed by one sampling period, the time when highest values of aconitate and C—A were observed.

## CONCLUSION

This study indicated that although the potential for increased forage and livestock-carrying capacity with N fertilization is tremendous, N fertilization may have adverse effects on Mg availability to ruminants. Nitrogen fertilization increased total N, K, K/C and N/TWSC in the forage. As K/C increased, the Mg/C and Ca/C ratios decreased, the K/(Ca + Mg) ratio increased, and concentrations of aconitate, C—A, and N/TWSC ratio increased. All these changes increased the potential tetany proneness of the forage.

Only Lincoln bromegrass was considered in this study and we do not know whether this variety and species is representative of cation and organic anion content responses of other forages. Methodology in this study evaluated forage that was progressively more mature at each sampling, rather than frequently clipped or continuously grazed by livestock. Therefore, we do not know how characteristic these bromegrass chemical data are compared to bromegrass chemical composition under grazing. Also, corroborating livestock data are vitally needed since sublethal effects of chemical composition of forage on livestock performance as well as absolute criteria are still rather evasive. However, realization of a potential tetany hazard and when it may occur is very important. Management practices are needed which will take advantage of the high yields resulting from N-fertilization while minimizing tetany hazards to livestock. It may be necessary to increase the Mg intake of cattle by oral supplementation or by applying MgO to the foliage in the field. Tetany-susceptible animals, like older lactating cows or those near calving, could not be grazed on N-fertilized pastures while hazards are high. Cutting of fertilized bromegrass for hay may be preferable to grazing. Certainly, many management practices can be devised to reduce tetany hazards while increasing yields by N fertilization.

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